

between 560 nm and 610 nm and are imaged by imaging lenses 40a and 40b near vertical optic axis 257 so as to fall on a yellow light receiving region toward the center of TDI detector 44.

In a manner similar to dichroic filters R, O, and Y, dichroic filters G and B are configured and oriented so as to image green and blue light wavebands onto respective green and blue light receiving regions of TDI detector 44, which are disposed toward the left-hand side of the TDI detector. By stacking the dichroic filters at different predefined angles, spectral dispersing filter assembly 252 collectively works to focus light within predefined wavebands of the light spectrum onto predefined regions of TDI detector 44.

Those of ordinary skill in the art will appreciate that the filters used in the spectral dispersing filter assembly 252 may have spectral characteristics that differ from those described above and in FIGURE 12. Further, the spectral characteristics may be arbitrary and not limited to dichroic in order to achieve the desired dispersion characteristics.

The wedge shape of the dichroic filters in the preceding discussion allows the filters to be placed in near contact, in contact, or possibly cemented together to form the spectral dispersing filter assembly 252. The angle of the wedge shape fabricated into the substrate for the dichroic filter allows easy assembly of the spectral dispersing filter assembly 252, forming a monolithic structure in which the wedge-shaped substrate is sandwiched between adjacent dichroic filters. If the filters are in contact with each other or cemented together, the composition of the materials that determine the spectral performance of the filter may be different from those which are not in contact. Those of ordinary skill in the art will appreciate that flat, non wedge-shaped substrates could be used to fabricate the spectral dispersing filter assembly 252. In this case another means such as mechanically mounting the filters could be used to maintain the angular relationships between the filters.

In addition to the foregoing configuration, non-distorting spectral dispersion system 250 may optionally include a detector filter assembly 254 to further attenuate undesired signals in each of the light beams, depending upon the amount of rejection required for out-of-band signals. FIGURE 13 illustrates the construction of an exemplary detector filter 254 corresponding to the five color bands discussed above and includes a blue spectral region 256, a green spectral region 258, a yellow spectral region 260, an orange spectral region 262, and a red spectral region 264, all of which are disposed side-by-side, as shown in the Figure. The corresponding spectral characteristics of the blue, green, yellow, orange, and red spectral regions or wavebands are respectively shown in FIGURES 14A-14E. The detection filter assembly shown in FIGURE 13 may be constructed by cementing separate filters in side-by-side

arrangement on a common substrate or by other means well known to those of ordinary skill in the art. Additionally, the ordinary practitioner in the art will understand that the filter may alternatively be placed at an intermediate image plane, instead of directly in front of TDI detector 44.

5 In the embodiment shown in FIGURE 11, light may pass through each dichroic filter in the spectral dispersing filter assembly 252 twice before exiting the spectral dispersing filter assembly 252. This condition will further attenuate out-of-band signals, but will also attenuate in-band signals. FIGURE 15 illustrates a third embodiment useful in the present invention in which the light does not pass through
10 another dichroic filter after reflection. In this embodiment, a plurality of cube dichroic filters, including a red cube filter 266, a yellow cube filter 268, a green cube filter 270, and a blue cube filter 272 are spaced apart sufficiently to ensure that light does not pass through any of the cube filters more than once. As with the second embodiment of FIGURE 11, the cube dichroic filters are oriented at appropriate angles to image light
15 within a predefined bandwidth to distinct regions on a TDI detector 274. As the light is reflected from each of cube dichroic filters 266, 268, 270 and 272, it is directed toward imaging lenses 40a and 40b, and different bandpass portions of the light are focussed upon corresponding red, yellow, green, and blue light receiving segments or regions defined on a light receiving surface of TDI detector 274. If desired, an optional
20 detector filter assembly 276 of similar construction to detector filter assembly 254 (but without the orange spectral region) may be used to increase the rejection of out-of-band signals. It should be apparent to those skilled in the art that separate spaced apart plates, or pellicle beam splitters could also be used in this application instead of the cube filters.

25 In the third embodiment illustrated in FIGURE 15, the image lenses 40a and 40b must be placed a sufficient distance away from the plurality of cube filters to minimize the clear aperture requirement for lenses 40a and 40b. Those skilled in the art will appreciate the clear aperture in the plane orthogonal to the page can increase as the distance between the lenses and plurality cube filters increases. Therefore, the
30 placement of lenses 40a and 40b must be chosen to appropriately accommodate the clear aperture in both planes.

The foregoing descriptions of the preceding non-convolving embodiments illustrate the use of four and five color systems. Those skilled in the art will appreciate that a spectral dispersing component with more or fewer filters may be used in these
35 configurations in order to construct a system covering a wider or a narrower spectral region, or different passbands within a given spectral region. Likewise, those skilled in the art will appreciate that the spectral resolution of the present invention may be

increased or decreased by appropriately choosing the number and spectral characteristics of the dichroic and or bandpass filters that are used. Furthermore, those skilled in the art will appreciate that the angles or orientation of the filters may be adjusted to direct light of a given bandwidth onto any desired point on the TDI detector.

5 In addition, there is no need to focus the light in increasing or decreasing order by wavelength. For example, in fluorescence imaging applications, one may wish to create more spatial separation on the TDI detector between the excitation and emission wavelengths by changing the angles at which the filters corresponding to those wavelengths are oriented with respect to the optic axes of the system. Finally, it will be
10 clear to those skilled in the art that dispersion of the collected light may be performed on the basis of nonspectral characteristics, including angle, position, polarization, phase, or other optical properties.

FIGURE 17 illustrates the images projected onto a detector for the present spectral decomposition embodiment in the case where three beads are in view. In this
15 illustration, each bead has a series of four unique reporters visible in which the reporters are constructed of up to four distinct fluorochromes. There is no spreading of the reporter images, as occurs in the prism based spectral decomposition embodiment. The field angle orthogonal to flow in object space is also indicated on FIGURE 17. In this particular configuration, the field angle in object space is less than $\pm 0.25^\circ$. Those
20 skilled in the art will appreciate that the field angle can be made larger or smaller. To the extent that the field angle is made larger, for example, to image cells over a wider region on a slide or in a broad flat flow, the field angle at the detector will increase in proportion to the number of colors used. Those skilled in the art will appreciate that broad flat flow can easily be created using commercially available flow cells as shown
25 in FIGURE 16B containing flow cell 290, with a cross section that is elongated in an axis perpendicular to both the flow and optical axes. The generation of broad flat flow is discussed in many references including U.S. Patent No. 5,422,712.

Fourth Embodiment of Apparatus for Spectral Decomposition and Imaging

The fourth embodiment of apparatus usable for spectral decomposition and
30 imaging in connection with the present invention, which is illustrated in FIGURE 16A, is similar to the second and third embodiments in that no convolution of the emission spectra with the image occurs as a result of the spectral decomposition process. Spectral decomposition occurs in an axis generally perpendicular to flow through the use of dichroic filters as previously described. However, in this embodiment, separate
35 imaging lenses and detectors are used for each spectral region. Dichroic filters 277 are placed at infinity (with respect to the object) after a collection lens 278 to minimize optical aberrations. After each dichroic filter 276a separate imaging lens 280 is used to